

CLAIMS

1. A method for detecting contaminating microbes possibly present in a blood product comprising blood cells comprising:

- a) subjecting a sample of the blood product to an aggregation treatment of the blood cells,
- b) substantially eliminating aggregates formed in step (a) by passage of the sample over a first filter allowing passage of contaminating microbes, but not cell aggregates,
- c) selectively lysing residual cells of the filtrate obtained in step (b),
- d) recovering the contaminating microbes by passage of the lysate from step (c) over a second filter allowing passage of cellular debris,
- e) adding a marker agent of the contaminating microbes either during step (a) or step (c), and
- f) analyzing material on the second filter to detect labeled contaminating microbes possibly retained by the second filter.

2. The method according to claim 1, further comprising addition of a permeabilization agent of the contaminating microbes in at least one of the steps (a), (c) or (e).

3. The method according to claim 2, wherein the permeabilization agent is selected from the group consisting of polyethylene imine, chlorhexidine diacetate, chlorhexidine digluconate, ethylene diamine tetraacetate acid (EDTA) alone or in combination with nisin, a detergent and mixtures thereof.

4. The method according to claim 3, wherein the detergent is selected from the group consisting of N-octyl  $\beta$ -D-glucopyranoside, SDS, Tween, triton, Brij and mixtures thereof.

5. The method according to claim 1, wherein the marker agent is a marker solution selected from among the group consisting of an esterase substrate, a labeled antibody and a marker of nucleic acids.

6. The method according to claim 1, wherein the marker agent comprises a fluorescent marker or an agent coupled to a fluorochrome or an enzyme enabling degradation of a substrate thereby made fluorescent.

7. The method according to claim 6, wherein fluorescence is detected by an excitation laser.

8. The method according to claim 1, wherein the blood cells of the blood product are platelets or red cells or a mixture thereof.

9. The method according to claim 1, wherein the blood product comprises platelets and step (a) comprises bringing the sample into contact with an aggregation composition comprising at least one aggregation agent selected from the group consisting of 1) a specific antibody of a platelet antigen, 2) a strong agonist of platelet activation selected from the group consisting of thrombin, TRAP (thrombin receptor activating peptide), trypsin, collagen, thromboxane A<sub>2</sub>, PAF (platelet activating factor), ionophore A23187, immune complexes and complement factors, and 3) a weak agonist of platelet activation selected from the group consisting of ADP, adrenalin, arachidonic acid, Von Willebrand factor, serotonin and epinephrine.

10. The method according to claim 9, wherein concentration of the antibody is between about 0.5 µg/ml and about 50 µg/ml.

11. The method according to claim 9, wherein concentration of strong agonist is between:

about 0.5 IU/ml and about 100 IU/ml for thrombin;

about 5 µM and about 200 µM for TRAP;

about 1 nM and about 500 nM for trypsin;

about 0.05 µg/ml and about 50 µg/ml for collagen;

about 0.01 µg/ml and about 5 µg/ml for thromboxane A<sub>2</sub>;

about 0.005 mg/ml and about 1 mg/ml for PAF; or

about 0.1 µM and about 100 µM for ionophore A23187.

12. The method according to claim 9, wherein concentration of the weak agonist is between:

about 0.5  $\mu$ M and about 100  $\mu$ M for ADP, adrenalin or epinephrine;

about 0.001 mM and about 10 mM for arachidonic acid;

about 0.001 mg/ml and about 1 mg/ml for Von Willebrand factor; or

about 0.05  $\mu$  and about 100  $\mu$ M for serotonin.

13. The method according to claim 9, wherein the antibody is selected from the group consisting of an anti-CD, anti-CD32, anti-PTA1, anti-D42, anti-GpIIb/IIIa and anti-GpIV antibody.

14. The method according to claim 1, wherein the blood product comprises red cells and step (a) comprises bringing the sample into contact with an agglutination composition comprising at least one agglutination agent selected from the group consisting of lectins, polyethylene imine, polyvinylpyrrolidone (PVP), gelatins, dextrans and polyethylene glycols (PEG).

15. The method according to claim 14, wherein the lectins have erythroagglutinin activity.

16. The method according to claim 14, wherein the lectins are selected from the group consisting of *Phaseolus vulgaris*, *Vicia sativa*, *Vicia faba* and *Erythrina corallodendron*.

17. The method according to claim 16, wherein concentration of *Phaseolus vulgaris* lectin is between about 10 µg/ml and about 200 µg/ml.

18. The method according to claim 14, wherein concentration of polyethylene imine is between about 0.1% (weight/volume) and about 40% (weight/volume).

19. The method according to claim 14, wherein the polyvinylpyrrolidone (PVP) is selected from the group consisting of PVP-40 and PVP-360 at a concentration between about 0.1% (weight/volume) and about 40% (weight/volume).

20. The method according to claim 14, wherein the gelatin is at a concentration between about 0.5% (weight/volume) and about 40% (weight volume).

21. The method according to claim 14, wherein dextran is selected from the group consisting of Dextran 70, Dextran 100 and Dextran 500 at a concentration between about 0.1% (weight/volume) and about 40% (weight/volume).

22. The method according to claim 14, wherein the polyethylene glycol is selected from the group consisting of PEG8, PEG17 and PEG35 at a concentration between about 0.05% (weight/volume) and about 40% (weight/volume).

23. The method according to claim 1, wherein step (c) is performed with a lysis solution comprising one or more detergents selected from the group consisting of saponin, SDS, Tween 20, Triton X100, Brij 96, Polidocanol, N-octyl  $\beta$ -D-glucopyranoside and sodium carbonate.

24. The method according to claim 1, wherein the contaminating microbes are aerobic or anaerobic bacteria, molds, yeasts, or live and/or dead bacterial spores.

25. The method according to claim 1, wherein the size of pores of the first filter are between about 2  $\mu\text{m}$  and about 20  $\mu\text{m}$ .

26. The method according to claim 1, wherein the size of pores of the second filter are between about 0.2  $\mu\text{m}$  and about 2  $\mu\text{m}$ .

27. The method according to claim 1, wherein the contaminating microbes are aerobic or anaerobic bacteria, molds, yeasts, or live and/or dead bacterial spores, and the size of pores of the first filter are between about 2  $\mu\text{m}$  and about 20  $\mu\text{m}$ , and the size of pores of the second filter are between about 0.2  $\mu\text{m}$  and about 2  $\mu\text{m}$ .

28. The method according to claim 1, wherein step (f) is performed in an enclosed device.

29. A device for concentrating contaminating microbes possibly present in a blood product comprising blood cells comprising:

a first watertight, sterile tank containing at least one blood cell aggregation agent and, optionally, at least one agent for labeling pathogenic microbes;

a second watertight, sterile tank containing at least one lysis agent for blood cells and, optionally, at least one agent for labeling pathogenic microbes;

a first filter located between the first and second tanks and capable of retaining aggregates formed in said first tank;

a second filter located downstream of the second tank and capable of retaining possible contaminating pathogenic microbes; and

a watertight, sterile connector placed between the first tank and the first filter, between the first filter and the second tank, and between the second tank and the second filter.

30. The device according to claim 29, further comprising a watertight, sterile connector to connect a bag containing the blood product to the first sterile tank.

31. The device according to claim 30, wherein the watertight, sterile connection connecting the bag containing the blood product to the first sterile tank has a reverse lock valve.

32. The device according to claim 29, further comprising means for sampling a determined volume of the blood product directly from a storage bag of the product into the first tank.

33. The device according to claim 30, wherein the first sterile tank is fitted with a sample suctioning system.

34. The device according to claim 33, wherein the suctioning system is a piston.

35. The device according to claim 29, wherein the second filter is enclosed in a membrane support having two parts that can be separated for removing the filter.

36. The device according to claim 29, which is enclosed and sterile.